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Properties and *in-vitro* test for antimicrobial activity of rice starch based biodegradable film Reinforced with magnesium oxide nanoparticle

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Abstract
In the present study, rice starch (RS) based film reinforced with magnesium oxide (Mgo) nanoparticle (NP) was developed at different plasticizer levels (glycerol 50, 60,70 g /100g of starch) and Mgo NP concentrations (0.1%, 0.3%, 0.7%, 1% and 1.3%) by casting a film forming solution on levelled teflon trays to improve the properties of starch based biodegradable films. Properties and *in-vitro* test of antimicrobial activity of Mgo/RS composite film were evaluated. Thickness, moisture and solubility of film were increased with higher level of plasticizer and increased concentration of magnesium oxide slightly increased the thickness and mechanical properties Mgo /RS composite film. Water vapour permeability (WVP) of film was lower with the increased plasticiser levels but improved with a reinforcement of Mgo nanoparticles.

Keywords: Rice starch, biodegradable, magnesium oxide, nanoparticles, reinforcement, mechanical properties

Introduction
Biodegradable polymers represent a growing field. In the food packaging sector, starch-based material has received great attention owing to it's biodegradability (Avella *et al.,* 2005). Rice starch has been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, as well as possessing good mechanical properties. Unfortunately, so far the use of biodegradable films for food packaging has been strongly limited because of the poor barrier properties and weak mechanical properties shown by natural polymers. For this reason Magnesium oxide (Mgo) nanoparticle was used to improve the properties of film. Magnesium oxide (Mgo) also known as magnesia, is a white hygroscopic solid mineral that occurs naturally as periclase. It has many advantages such as good reproducibility, suitability for large-scale production, simplicity and low cost. It is used as nanofiller to improve the properties of materials and it also contains good antibacterial properties (Kavitha *et al.,* 2013: Xie *et al.,* 2012) [11, 18]. The application of nano-composites promises to expand the use of edible and biodegradable films that reduce the packaging waste associated with processed foods and this supports the preservation of fresh foods by extending their shelf life (Sorrentino *et al.,* 2007) [16].

Materials and Methods
Materials: For present study, Mgo nanoparticles were procured from Reinste Nano Ventures Private limited, New Delhi.

Film preparation
Mgo reinforced rice starch films was prepared with modifications to the method described by Bajpai *et al.* (2011) [2]. A film forming dispersion was prepared using 3 g of starch and 100 ml distilled water. The plasticizer was added at different concentration of 50, 60, 70 g glycerol/100 g starch. The pH of film forming solution was adjusted to 10 with NaOH. Then the solution was heated to 90 ± 2 ºC for 30 min in a water bath while being stirred continuously. The solution was then filtered through two layers of muslin cloth to remove any coagulation. Subsequently, Mgo at various concentrations (0, 0.4, 0.7, 1 and 1.3 g/ 100ml of solution) were added and stirred for 20 min and cost onto flat. Leveled non-stick tray to set. Once set, the trays were held overnight at 55 °C for 10 h undisturbed, and then cooled to an

ambient temperature before peeling the films off the plates. The film samples were stored in plastic bags and held in desiccators at 60% RH for further testing. All treatments were made in triplicate.

Physical and mechanical properties of developed packaging film

Film thickness: Thickness of the developed packaging film was determined using a precision digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan) at five random locations of the film and average values were computed.

Moisture content: The moisture content was determined by placing the films in an oven (Narang Scientific Works, New Delhi, India) at 110 ˚C until constant weight was achieved. The moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Film solubility: The solubility of films was determined as per the method described by Ghasemlou *et al.* (2011). The dried films were immersed in 50 mL of distilled water for 12 h at 25 ˚C. Next, the samples were taken out from the water by blotting with filter papers and weighed to remove the surface adsorbed water. Remaining pieces of films were dried at 110 ˚C to constant weight and solubility was calculated using following equation:

$$\text{WS (\%)} = \frac{\text{Initial dry weight} - \text{final dry weight}}{\text{Initial weight}} \times 100$$

Tensile strength (TS): The films with known thickness were cut in strips (50 x 50mm) and mechanical properties were studied using a TA.TX2 texture analyzer (Stable Microsystems, Surry, UK). The mechanical properties were determined according to ASTM D638-03 method (2003). The pieces of films were clamped between two jaws with an initial distance of 40 mm. Each film deformed under a tensile load with a cross-head speed of 1 mm/s until the sample was broken and Force (N) and deformation (mm) were recorded during extension of films and force- deformation curve was obtained. The tensile strength and elongation at break were calculated using following equations:

$$E = \frac{dr - do}{do} \times 100$$

$$TS = \frac{Fm}{A}$$

Where d_r was the distance between the grips holding the specimen before breaking and d_o is the distance between the grips holding the specimen after breaking. Fm is the maximum force (N) and A is the area of film cross-section (thickness x width, m^2).

Water vapor permeability (WVP): Water vapor permeability (WVP) is a proportional constant assumed to be independent of the water vapor pressure gradient applied across the film. WVP was determined according to ASTM E 96 using aluminium cups. 50 cm^2 diameter of sample were sealed on the aluminium cups containing fused $CaCl_2$ which was highly hydroscopic. Samples were placed on the aluminum cups and 50 cm^2 surface areas sealed using hot wax. Before placing the samples in humidity chamber, they were rested at room temperature and weighed. The sealed cups were kept in chamber at 38 to 39 °C in 90% RH. The samples are checked for gain in weight at fixed interval. The gain in weight was due to the absorption of moisture by $CaCl_2$ which permeated through the film. Weight gain was plotted against time and linear least-square method used to calculate WVTR using the following formula. Water vapour transmission rate (WVTR) = Slope × area of sample gm/m^2 /day

***In vitro* test of film for antimicrobial activity:** The antimicrobial activity of Mgo incorporated packaging film was assessed according to Chawengkijwanich *et al.* (2008) [3]. Test pieces (a square of 5 cm) of Mgo incorporated starch based film and pure starch based film were placed in sterilized petri dishes under aseptic conditions. One ml of the *E. coli* solution was pipetted onto each test piece in its petri dish and inoculated with *E. coli* were placed under light. Samples were taken in three replicates at 60 min intervals for 3 h. After the sample test piece was removed from the light, it was immediately washed, placed in a sterile cup with 9 ml of sterile deionized water, and shaken for 10 min. One milliliter of solution was withdrawn at each sampling and diluted to 1/10, 1/100, 1/1000, and 1/10,000 with sterile distilled water. Three replicated plates were used for each solution. The plates were incubated for 24 h at 37 °C, and the colony-forming units (CFU) were then counted and recorded.

Result and discussion

The effects of plasticizer concentrations and Mgo nanoparticle concentrations on thickness of film

The effects of plasticizer concentrations (50, 60, 70 g glycerol/100 g starch) and Mgo nanoparticle concentrations (0, 0.4, 0.7, 1 and 1.3 g/ 100ml of solution) on thickness of rice starch based packaging film are presented in table 1. From the Table, it is observed that, the increase in glycerol concentration used as plasticizer increased the thickness of the film significantly and also increased in concentration of nanoparticle was also found to be slightly increased thickness from 0.152 to 0.157 at concentration of 50g g glycerol/100 g starch, 0.178 to 0.198 mm and 0.195 to 0.214mm at concentration of 60 and 70 g glycerol/100 g starch. The similar effect on thickness of film was reported by Kadam *et al.* (2013) [10] and Zhou *et al.* (2013). According to Embuscado and Huber (2009) [6], biodegradable films usually have thickness of less than 0.300 mm.

The effects of plasticizer concentrations and Mgo nanoparticle concentrations on moisture content (MC) of film

The effects of plasticizer concentrations (50, 60, 70 g glycerol/100 g starch) and Mgo concentrations (0, 0.4, 0.7, 1 and 1.3 g/ 100ml of solution) on moisture content (MC) of rice starch based nano functionalized packaging film are presented in table 2. Increased plasticizer concentrations (50, 60, 70 g glycerol/100 g starch) was increase the moisture content of film significantly from 32.53 to 37.43% at controlled sample. Moisture content is a parameter related to the total void volume occupied by water molecule in the network microstructure of the film. No regular variation of MC was seen with the different Mgo concentrations which suggested that present Mgo amount (≤1.3%) were insufficient to alter MC of the film. Similar results were noticed by Yanxia *et al.* (2011) [19].

The effects of plasticizer concentrations and Mgo nanoparticle concentrations on solubility of film

The effects of plasticizer concentrations (50, 60, 70 g glycerol/100 g starch) and and Mgo nanoparticle concentrations (0, 0.4, 0.7, 1 and 1.3 g/ 100ml of solution) on solubility of rice starch based nano functionalized packaging film are presented in table 3. The increased plasticizer concentration higher the solubility of film. The similar effect was noticed by Colussi *et al.* (2014) [5]. Solubility values were lower than the results obtained by Mehyar and Han (2014) that studied rice starch film and found 44% of water solubility. Plasticizers promote a decrease in intramolecular force and the network becomes less dense thus improving flexibility and extensibility of film. No regular variation was observed in change in different concentration of Mgo nanoparticle. Similar results were noticed by Yanxia *et al.* (2011) [19]. Addition of nanoparticle alters the structure of film. Higher concentration of Mgo nanoparticle implies that the large water insoluble Mgo agglomerates were blocking the micro-path in the network microstructure.

The effects of plasticizer concentrations and Mgo nanoparticle concentrations on Tensile strength (Mpa) of film

The effects of plasticizer concentrations (50, 60, 70 g glycerol/100 g starch) and quantity of Mgo nanoparticle (0, 0.4, 0.7, 1 and 1.3 g/ 100ml of solution) on tensile strength of rice starch based packaging film are presented in table 4. From the table 4, it is observed that increase in plasticizer level (50 – 70 %) had lower tensile strength (3.31- 1.58 Mpa) respectively, when compare with the Mgo added film. The similar results were noticed by Colussi *et al.* (2014) [5]. Glycerol and starch polymers presented a strong interaction and at low glycerol content (10%) resulted in more brittle in film structure were reported by Myllarinen *et al.* (2002) [14]. The tensile strength of cast film increased as increased in Mgo nanoparticle (NP) concentration it might be due to more possible interaction that involves electrostatic attraction between negatively charged and positively charged molecular complex during the preparation of film solution. Similar results have been reported by Zhou *et al.* (2009) [20] and Guerrero *et al.* (2010) [9].

The effects of plasticizer concentrations and Mgo NP concentrations on elongation (%) of film

The rice starch based films prepared with different concentration of plasticizer and TiO_2 were presented in the table. From the table it is observed that elongation of film was significantly increased with increased in concentration of glycerol and nanoparticle. This might be due to ability of glycerol to reduce interactions between polymer chains, thereby increasing flexibility (Sothornvit and Krochta, 2000) [17]. Mali *et al.* (2004) reported that glycerol concentration is a important factor for altering the profile of mechanical properties of starch films and with increased in plasticizer level increases the flexibility. The increased in nanoparticle concentration had higher elongation. It might be due to the strong chain and polar interaction between side chains of starch molecules occurred which restricted segment rotation and molecular mobility. This might have lead to increased elongation. The results found were similar to the findings of Guerreo *et al.* (2010) [9]. The increased concentration of nanoparticles with constant starch concentration increases the elongation of film. The similar results have been reported by Zhou *et al.* (2009) [20].

The effects of plasticizer and Mgo nanoparticle concentrations on water Vapour permeability (g/m^2/day) of film

Water vapour permeability (WVP) is the ratio of water vapour transmission through unit area of the material of a certain thickness, induced by the difference in vapour presser between specific surfaces under defined temperature conditions (ASTM E96-00, 2000). With the addition of 70% glycerol the WVP increased significantly. It mignt be due to glycerol binds to biopolymer molecules decreasing the mobility of the molecules, facilitating permeation. The similar results were reported by MCHugh and Krochta (1994) [13]. Kadam *et al.* (2013) [10] reported that higher WVP is one of the major limitations when using biodegradable film as food packaging material. Glycerol has many –OH groups, which has strong absorption ability. Thus, the decrease in absorption ability of WVP of the film was observed. The effects of quantity of nanoparticles on WVP of starch based packaging film are presented in table 6. From the table, it is found that as the quantity of nanoparticles increased, WVP of starch based film decreased. It might be due to the distribution of nanoparticles in the film forming solution. The results are in line with the findings of Quingshen *et al.* (2009) [15].

***In vitro* test for antimicrobial activity of Mgo nanoparticle added packaging film**

The effect of Mgo added starch based films was determined at 0th h, after 1, 2, 3 and 4 h. The growth of *E. Coli* decreased from 160×10^2 to 12×10^2 in Mgo incorporated film and also there was a decrease in growth of *E-Coli* in pure rice starch film (without nanoparticles) from 163×10^2 to 92×10^2. There was a significantly more reduction in growth of organism at Mgo incorporated film compared to the pure starch film. It might be due to *E. coli* was killed by contact with a Mgo surface might have play a role in inactivating microorganisms by oxidizing the polyunsaturated phospholipid component of the cell membrane of microbes. The similar results have been reported by Fujishima *et al.* (1972) [7]; Manees *et al.* (1999) [12]; Cho *et al.* (2004) [4].

Table 1: Thickness of rice starch based film prepared with different glycerol and nanoparticle concentration

Nanoparticle concentrations (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	0.152 ± 0.01^{a}	$0.178 \pm 0..01^{e}$	0.195 ± 0.01^{i}
0.1	0.153 ± 0.01^{ab}	0.179 ± 0.02^{ef}	0.197 ± 0.01^{ij}
0.3	0.154 ± 0.01^{bc}	0.181 ± 0.02^{f}	0.202 ± 0.01^{k}
0.7	0.155 ± 0.01^{bcd}	0.187 ± 0.01^{g}	0.209 ± 0.01^{l}
1	0.156 ± 0.01^{cd}	0.192 ± 0.01^{h}	0.212 ± 0.01^{m}
1.3	0.157 ± 0.01^{d}	0.198 ± 0.01^{j}	0.214 ± 0.01^{m}

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 6.07E-05

Table 2: Moisture content of rice starch based film prepared with different glycerol and nanoparticle concentration

Nanoparticle concentrations (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	32.53 ± 0.2 [a]	35.34 ± 0.9 [bc]	37.43 ± 0.7[def]
0.1	31.98 ± 0.7[a]	34.62 ± 0.7[b]	36.71 ± 0.2[cd]
0.3	34.763 ± 0.4[b]	37.53 ± 0.4[def]	36.82 ± 0.9[cd]
0.7	36.66 ± 0.9[cd]	38.46 ± 0.8[ef]	38.88 ± 0.3[f]
1	35.35 ± 0.7[bc]	36.32 ± 0.1[cd]	37.65 ± 0.5[def]
1.3	36.34 ± 0.2[bc]	37.96 ± 0.9[de]	38.45 ± 0.2[ef]

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 23.5

Table 3: Solubility of rice starch based film prepared with different glycerol and nanoparticle concentration

Nanoparticle concentration (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	19.27 ± 1.0[fg]	19.98 ± 0.3[gh]	20.85 ± 0.9[h]
0.1	18.35 ± 0.2[ef]	19.24 ± 0.2[fg]	21.24 ± 0.2[h]
0.3	17.47 ± 0.2[cde]	18.24 ± 0.8[ef]	20.04 ± 0.02[gh]
0.7	16.23 ± 0.01[bc]	17.12 ± 1.0[cde]	18.36 ± 0.8[ef]
1	15.49 ± 0.5[ab]	16.65 ± 0.9[bcd]	17.24 ± 1.5[def]
1.3	14.03 ± 2.0[a]	15.32 ± 0.7[ab]	16.24 ± 0.4[bc]

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 25.9

Table 4: Tensile strength (Mpa) of rice starch based film prepared with different glycerol and nanoparticle concentrations

Nanoparticle concentrations (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	3.31 ± 0.1[d]	2.35 ± 0.1[b]	1.58 ± 0.2[a]
0.1	5.53 ± 0.01[g]	3.83 ± 0.01[e]	2.63 ± 0.02[c]
0.3	7.67 ± 0.01[j]	6.85 ± 0.01[h]	4.76 ± 0.01[f]
0.7	9.94 ± 0.01[l]	7.23 ± 0.01[i]	5.55 ± 0.01[g]
1	11.34 ± 0.09[m]	9.46 ± 0.02[k]	7.74 ± 0.02[j]
1.3	12.68 ± 0.11[n]	10.05 ± 0.02[l]	9.43 ± 0.01[k]

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 0.28

Table 5: Elongation of rice starch based film prepared with different glycerol and nanoparticle concentrations

Nanoparticle concentrations (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	26.4 ± 2.0[a]	34.4 ± 2.0[d]	46.2 ± 1.2[i]
0.1	30.4 ± 0.1[b]	38.8 ± 0.9[f]	50.4 ± 0.3[j]
0.3	32.2 ± 0.3[c]	42.6 ± 0.3[g]	56.6 ± 0.4[l]
0.7	36.6 ± 0.05[e]	50.8 ± 0.3[j]	65.5 ± 0.2[o]
1	44.2 ± 0.1[h]	59.2 ± 0.3[m]	70.8 ± 0.3[p]
1.3	38.6 ± 0.1[f]	54.4 ± 0.2[k]	62.6 ±0.2[n]

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 22.7

Table 6: Water vapour permeability of rice starch based film prepared with different glycerol and nanoparticle concentrations

Nanoparticle concentrations (%)	Glycerol (g /100 g starch)		
	50 g	60 g	70 g
0	105.28 ± 1.0[d]	116.25 ± 1.8[h]	127.03 ± 0.04[k]
0.1	104.92 ± 1.0[d]	114.83 ± 0.01[h]	125.84 ± 1.9[k]
0.3	103.71 ± 1.0[d]	112.94 ± 0.06[g]	123.12 ± 1.2[j]
0.7	99.83 ± 0.02[c]	109.02 ±0.1[f]	119.43 ± 0.4[i]
1	96.03 ± 1.0[b]	107.14 ± 0.02[e]	115.26 ± 0.8[h]
1.3	92.64 ± 0.03[a]	103.82 ± 0.9[d]	112.92 ± 0.1[g]

[a] Different letters in the same column differ statistically (p<0.05). R^2 value: 29.9

Table 7: Effect of Mgo on microbial growth (TPC)

Time	Rice starch film	Mgo incorporated RS film
0^{th} h	$163 \times 10^2 \pm 18^e$	$160 \times 10^2 \pm 13^e$
After 1 h	$146 \times 10^2 \pm 15^e$	$102 \times 10^2 \pm 11^{cd}$
After 2 h	$128 \times 10^2 \pm 15^{de}$	$78 \times 10^2 \pm 7b^c$
After 3 h	$103 \times 10^2 \pm 8^{cd}$	$53 \times 10^2 \pm 5^b$
After 4 h	$92 \times 10^2 \pm 7^{cd}$	$12 \times 10^2 \pm 4^a$

Conclusion

The addition of different levels of plasticize made increased thickness of films. Tensile properties of developed film were significantly increased with increasing Mgo nanoparicle concentrations (0.1% to 1%) but decreased with the increasing plasticizer levels (50g to 70g/ 100 g of starch). Moisture barrier and thermal properties of film were lower with the increasing plasticiser levels but improved with a reinforcement of Mgo nanoparticles. Antimicrobial activity of

film was a significantly more reduction in growth of organism at Mgo incorporated film compared to the pure starch film.

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